Genetic Mapping and Maps

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Abstract Early genetic analyses of maize were rooted in genetic mapping, and mapping continues to be an important tool for contemporary maize geneticists. Mapping is extraordinarily easy in maize; consequently many maps have been made. The first genetic map published for maize in 1935 contained 62 loci defined by morphological variants. Current genetic maps contain thousands of loci defined by morphological, biochemical, cytogenetic, and molecular polymorphic variants. These maps serve critically important functions in linking genes to traits, facilitating comparative evolutionary studies, enabling positional cloning, and anchoring the physical map for genome sequencing. Sequencing in turn now makes it possible to derive the map locations of sequenced genes by matching to genomic sequences that have been anchored to the physical map.

1 Definition

A genetic map, or linkage map, is a map of the frequencies of recombination that occur between markers on homologous chromosomes during meiosis. Recombination frequency between two markers is proportional to the distance separating the markers. The greater the frequency of recombination, the greater the distance between two genetic markers; conversely, the smaller the recombination frequency, the closer the markers are to one another. Thus a genetic map is a representation of recombination events and frequencies, rather than a physical map. The genetic and physical order is the same but distances are not. Although the *average* centimorgan

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is about 180 kb, physical distance is not consistently proportional to recombination frequency for each interval and varies widely along a chromosome (Wei et al., 2007).

2 Utility of Genetic Maps

Genetic maps provide a way to link a genetic region to a trait of interest. Mapping provides a mechanism to track the co-segregation of genetic markers with traits in segregating populations. Such marker tracking can be used in selection (marker-assisted selection) of genes responsible for agronomically important traits and thus serve as an aid in crop improvement (Morgante and Salamini, 2003; Tuberosa and Salvi, 2006).

Genetic maps can be used in comparative studies to understand the processes that led to the evolution and diversification of a species. Between related taxa, comparative mapping can reveal regions of chromosomal synteny or conservation of gene order; and within a taxon, mapping can pinpoint regions of chromosomal duplication derived from ancient polyploidization events (for example, Helentjaris et al., 1988; Moore et al., 1995; Bennetzen and Freeling, 1997; Devos and Gale, 1997; Feuillet and Keller, 2002; Wei et al., 2007).

High-resolution genetic maps are essential tools for positional cloning. Recombinations between markers flanking a cloning target localize the target with increasing precision, as closer mapped markers are incorporated in the analysis. The most tightly linked markers co-segregate with the target. Positional cloning has been used to isolate a number of maize genes in the past couple of years and is likely to see more use in the future (Bortiri et al., 2006a; Bortiri et al., 2006b; Chuck et al., 2007; Salvi et al., 2007).

Genetic maps serve as a foundation for anchoring the physical map. Assemblies of genome fragments are formed into physical contigs (contiguous sequences). The placement of those contigs to chromosomes, and their orientation and order on the chromosomes, are achieved by correlating to genetic maps, which are chromosome-based (Coe et al., 2002; Cone et al., 2002; Wei et al., 2007). A genetic-map skeleton of markers that are matched to the physical map serves as an invaluable aid for genome sequencing and assembly (Messing and Dooner, 2006).

3 Making a Map

The first two basic requirements for genetic mapping are: (a) parents that are polymorphic for measurable traits and detectable markers, and (b) a population segregating for the traits of interest, made by crossing the polymorphic parents. Maize is ideal for genetic mapping, as the vast amount of diversity among maize lines provides a rich source of polymorphisms in traits and markers. The separation of male and female flowers on the plant makes it extremely easy to make controlled crosses, and the large number of progeny kernels from each cross can provide an ample segregating population from a single ear. Moreover, because maize plants can be both outcrossed

and self-pollinated, making a mapping population segregating for the trait of interest is as simple as crossing two polymorphic parents and then self-pollinating the F1 to generate an F2, or crossing the F1 to one or both of the two parents to generate a backcross (BC) population.

3.1 Trait and Marker Polymorphisms

The first requirement for genetic mapping is to have parental lines with trait and marker polymorphisms. Maize has tremendous genetic diversity; surveys of diverse collections of maize lines have led to the estimate that the average maize gene contains about 200 nucleotide polymorphisms and 20-30 amino acid polymorphisms (Buckler et al., 2006). At least some of these molecular polymorphisms are likely to underlie diversity in function that is manifest as polymorphisms in trait expression.

Trait Polymorphisms

Hundreds of trait polymorphisms have been mapped in maize. These include: morphological traits with phenotypes explained by alternate alleles of a single gene, such as white/yellow endosperm, colored/colorless aleurone, dwarf/normal plant stature; and quantitative traits involving multiple genes controlling variation in agronomically important characteristics such as productivity; starch, oil, and protein composition of the kernel; and tolerance to biotic and abiotic stresses.

Marker Polymorphisms

Early maize mappers took advantage of isozyme variation and were able to map a large number of isozyme polymorphisms (for example, Wendel et al. [1988]). Nowadays, isozyme markers have been supplanted by DNA markers, which capitalize on the high level of variation in nucleotide sequence across maize lines. The five major types of molecular markers that have been used in maize mapping are restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), insertion-deletion polymorphisms (IDPs), and single nucleotide polymorphisms (SNPs).

RFLP polymorphisms are detected by digesting genomic DNA with restriction enzymes and then detecting the restriction fragments by DNA gel blot hybridization with a radioactive probe made from genomic DNA or cDNA. The high degree of nucleotide sequence polymorphism in maize means that digestion with only four to six restriction enzymes is usually sufficient to detect polymorphism between any two maize lines for any probe tested. This led to widespread use of RFLPs as one of the first molecular marker types for maize (Evola et al., 1986; Helentjaris et al., 1986; Burr et al., 1988; Gardiner et al., 1993). Drawbacks to RFLPs as markers are the labor intensive process involved in preparing the DNA blots and the need to use radioactivity to detect hybridization.

Several types of polymerase chain reaction (PCR)-based markers have been used for mapping genes in maize. Among these are AFLPs and amplified polymorphisms

associated with miniature inverted repeat transposable elements (MITEs). AFLPs are detected by digesting genomic DNA with restriction enzymes and then ligating adaptors to the ends of the fragments. Subsets of the restriction fragments can be amplified using primers complementary to the adaptor and the restriction site, and then the fragments are visualized on denaturing acrylamide gels (Vuylsteke et al., 1999). A similar technology was used to develop MITE-associated markers by including a primer complementary to the MITE inverted repeat in the PCR amplification reaction (Casa et al., 2000; Casa et al., 2004).

Probably the most widely used type of PCR-based marker is the SSR. SSRs are tandemly repeated mononucleotide, dinucleotide, trinucleotide or tetranucleotide sequences that are abundant and dispersed across the maize genome (Taramino and Tingey, 1996; Sharopova et al., 2002). SSR polymorphism arises from variation in the number of repeats at a given locus. This variation is detected by PCR using primers that flank the SSR and then fractionating the PCR products by gel electrophoresis to display length differences. The ease and relatively low cost of detection for SSRs makes them an attractive marker type for mapping.

IDPs result from insertions or deletions (InDels). Many IDPs for maize have been developed from InDels in introns or 3′ untranslated regions of transcribed genes (Bi et al., 2006; Fu et al., 2006). IDPs, like SSRs, are easily detected by PCR, using primers that flank the InDel, followed by gel electrophoresis to detect length differences.

SNPs are more abundant than the other types of polymorphisms; on average, between any randomly chosen pair of inbreds, there is one SNP in every 150 bp. Maize SNPs have been developed by comparative sequencing across 14 maize inbreds (Bi et al., 2006). For genotyping with SNPs, alleles can be discriminated by one of two basic approaches – PCR-based primer extension or differential hybridization – and allelic differences can be detected using mass spectrometry, fluorescence, or chemiluminescence methods (reviewed in Kim and Misra, 2007).

3.2 *Mapping Populations*

There are several types of mapping populations, each with its own advantages. Probably the most versatile population is an F2, as this kind of population can be produced promptly and is easy to analyze; individuals in the population will have one of three possible genotypes (two homozygous and the heterozygous genotypes). In backcross (BC) populations, there are only two possible genotypes (homozygous and heterozygous). Both F2 and BC populations lend themselves well to mapping of one or a few traits, especially if recessive individuals can be analyzed as a pool by bulked segregant analysis (Michelmore et al., 1991; Carson et al., 2004).

Two disadvantages of F2 or BC populations are that phenotypes of individuals in the population can only be scored in a single generation and seed for the population is limited. One way to overcome these difficulties is to self-pollinate the F2 plants to produce a population that can be analyzed as F3 families. Another method is to produce an immortalized F2 (IF2) by chain-pollinating (one male on one sib) and bulking seed within individual F3 families to "fix" the alleles of the F2 parent in

the progeny (Gardiner et al., 1993). This produces a larger store of seed, but requires further maintenance of the immortalized population, in which the advanced progenies may be subject to changes by genetic drift.

Two types of populations – doubled haploids and recombinant inbred lines (RILs) – circumvent the problem of limited seed, as both constitute permanent populations; as such, they can be used for assessing phenotypic variation through repeated measures across time and environment (McMullen, 2003). Doubled haploid populations are generated directly from F1 plants. Because they are homozygous, they effectively fix the linkage groups present in the gametes of the F1 and have the same mapping resolution as BC progeny (Snape, 1988). Homozygosity of these populations means that they can be easily maintained by sib- or self- pollinations.

RILs are made by repeatedly self-pollinating single-seed descendants of individuals from an F2 population to produce virtually complete homozygosity for linkage groups originally present in the F2 (Burr et al., 1988; Burr and Burr, 1991). Once homozygosity is attained, RILs can be perpetuated by sib- or self-pollination. The homozygous nature of the lines allows polymorphisms for presence *vs.* absence of a marker to be scored unambiguously. A number of maize RIL populations are publicly available (Maize Genetics Cooperation Stock Center; http://maizecoop.cropsci.uiuc.edu/). This enables multiple researchers to use the RILs; as a result, the genetic information gathered from mapping in RIL populations is cumulative. For codominant markers, the resolution of RILs is essentially equivalent over short intervals to that of F2s or IF2s, because F2 plants contain products from two distinct meioses and RILs accumulate a comparable number of recombination events during their derivation.

The mapping resolution of RILs can be increased by random intermating for one or more generations before the selfing rounds are begun. This strategy was used to create an intermated RIL (IRIL) population derived from crossing B73 and Mo17, self-pollinating the F1 and then randomly mating progeny for four generations before selfing by single-seed descent (Lee et al., 2002). The resulting Intermated B73-Mo17 (IBM) population has a very high mapping resolution, ~ 0.4 centimorgans (1 centimorgan = 1 map unit = 1% recombination). The IBM population (~302 IRILs, conveniently scored as a subset of 286 lines plus the two parent inbreds in three 96-well plates) is publicly available and has been used extensively for genetic mapping by many research groups. The resolution of the full population is sufficient to place on average about one recombination breakpoint within the length of a typical bacterial artificial chromosome (BAC) clone (~140-160 kb). Subsets of 94 lines, equivalent to about 750 tested gametes, can be used for approximate mapping, with the Community IBM Mapping utility (http://www.maizemap.org/CIMDE/cIBMmap.htm).

3.3 Collecting and Analyzing Data to Construct a Map

Once a population segregating traits of interest is obtained, mapping the trait typically involves measuring the phenotype and determining the genotype of each member of the population. Genotyping with the molecular markers used in current mapping is a two-step process. First, DNA samples from the parents of the mapping population

are screened for polymorphisms, using markers that span the chromosome(s) of interest. To scan the whole genome, polymorphic markers spaced approximately every 25-30 cM are needed. The second step is to use the polymorphic markers to determine the genotypes for each member in the population, or, in the case of bulked segregant analysis, for the pools of recessive and normal individuals. Cataloguing genotypes for large numbers of individuals and/or markers can be simplified using software specifically designed for collecting and managing mapping data (Sanchez-Villeda et al., 2003).

To construct the map, associations of genotype to phenotype must be derived. For bulked segregant mapping of a simple recessive trait, single-locus associations are made by comparing SSR or RFLP band intensity. Marker alleles linked *in cis* with the recessive trait allele will be overrepresented in the pool of homozygous recessive individuals and underrepresented in the pool of control individuals (Carson et al., 2004). Markers that show evidence of linkage can then be used to determine genotypes for individuals in the pools, and genetic distances can be estimated by calculating recombination frequencies.

For whole-genome mapping, genotype to phenotype correlations require more sophisticated computation. A number of mapping programs are available for mapping traits controlled by single genes, as well as quantitative traits. One of the first mapping packages, still in use, is MAPMAKER/EXP, which constructs genetic linkage maps using data generated from experimental populations (F2, BC and RIL [Lander et al., 1987; Lincoln et al., 1992]). (Note: For closely spaced markers in the IBM population, maps generated as RIL with MAPMAKER present distances approximately 4-fold greater than standard centimorgans [Winkler et al., 2003], an approximation that is useful for comparison with mapping data from other population types.) Other programs allow integration of data from different experiments (JoinMap; Stam, 1993) and adjustments in map distance due to the extra rounds of intermating in IRIL populations (IRILmap; Falque, 2005). Output from MAPMAKER/EXP can feed QTL mapping programs, such as MAPMAKER/QTL (Lincoln et al., 1992) and QTL Cartographer (Basten et al., 1997). Other QTL mapping programs bypass MAPMAKER and generate maps directly from input data (reviewed in Manly and Olson, 1999).

4 Maize Genetic Maps: Past and Present

Mapping in maize has a long history based on cumulative shared information that laid the foundation for modern molecular marker-based maps. The first full genetic maps were a part of the seminal monograph on maize genetics published by Emerson, Beadle, and Fraser in 1935. Data from individual gene-to-gene recombination experiments, made available by cooperating research scientists and collated by G. W. Beadle, were constructed into maps by M. M. Rhoades, which were published in the early issues of the Maize Genetics Cooperation Newsletter (MNL). These maps set the precedent for orientation with the cytologically short-arm end as the starting point, which depended on correlations of cytological data with the genetic

data (only chromosome 3 was mis-oriented in the original maps and was corrected soon after). New phenotypically defined genes and new gene-to-gene data were accreted on the 1935 skeleton for the next 70 years. A key innovation in maize mapping came with the division of each chromosome into "bins", which were defined by a set of "core" markers dispersed at regular intervals (Gardiner et al., 1993). The near-immediate impact of defining bins by flanking core markers was that many groups adopted the core markers in their various mapping experiments. As discussed below, when maps contain common markers, making linkages across those maps is possible.

As mapping continued, it soon became clear that genetic maps constructed from different mapping populations could show differences in the order and/or distances between genetic markers (for example, see Sharopova et al., 2002). In addition, some genetic markers proved to be present in some maize lines, but absent from others (Gardiner et al., 1993; Davis et al., 1999). The recent discovery of the Helitron class of transposons has shed light on these anomalies. Helitrons can carry genes or gene segments and can mobilize these gene segments in the genome (Lai et al., 2005; Morgante et al., 2005). As a result, chromosomal segments can exhibit non-colinearity, differing in marker order, distance between markers, or both.

Currently, over 150 maps – many targeted at mapping QTL – derived by over 40 research groups from 130 different mapping populations, are documented in the Maize Genetics and Genomics Database (MaizeGDB). The most current genetic map, IBM2 2005 Neighbors, is one of several maps based on the IBM IRIL population (Coe and Schaeffer, 2005). (A reference guide to the IBM maps is available at MaizeGDB [http://maizegdb.org/neighbors.php].) Fig. 1 and Table 1 highlight the expansion in marker density between the 1935 map and modern maps. The 1935 map contained only a few loci, most defined by morphological traits. By contrast, the IBM maps contain thousands of loci, which include both named genetic loci and loci defined by molecular markers not yet linked to genes. Inspection of a representative genetic interval on chromosome 2 – which includes *lg1*, *gl2* and *b1* – reveals that, as mapping information accrued and the number of loci found to lie in the *lg1-gl2* and *gl2-b1* intervals increased, the map distances in these intervals did not change from those established in 1942. This observation underscores the incredible accuracy of early mapping efforts.

5 Linking Genetic Maps to Other Maps

5.1 *Genetic to Genetic*

The key to linking genetic maps to one another is the use of common markers for mapping in different populations. If a marker unique to one map is located between common markers on the two maps, its location can be determined by extrapolating from the normalized distance between the common markers. This strategy has been

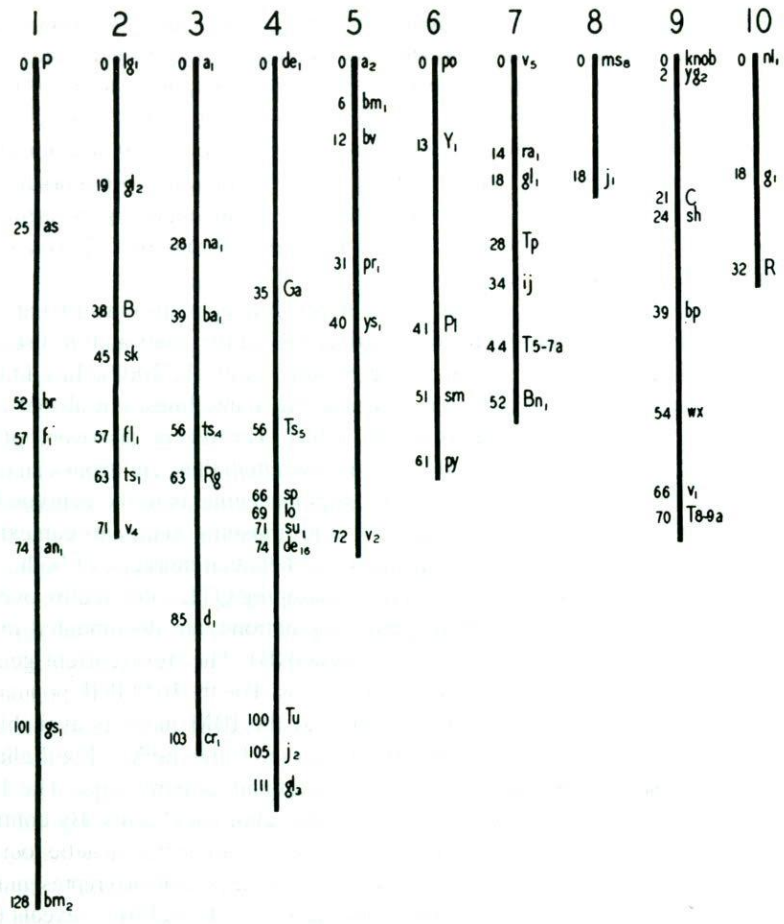

Fig. 1 Diagram of the first maize genetic map. Published in 1935 (Emerson et al., 1935), this map contained 62 loci defined by morphological variants, one cytological feature (the knob on the tip of chromosome 9) and two A-A translocation breakpoints (T5-7a and T8-9a)

applied over the years to generate a number of genetic-to-genetic map linkages, which have been published in various issues of the annual MNL. As marker numbers have increased, however, extrapolation through hand calculation has become extremely laborious. More recently, an algorithm was developed to link genetic maps and applied to great advantage to create the IBM2 Neighbors map series (Coe and Schaeffer, 2005). The most recent version, IBM2 2005 Neighbors, incorporates 14 genetic maps built on the frame of the IBM2. The nearly 35,000 loci represent the locations of markers mapped using either traditional genetic strategies or by placement to BAC clones (see below).

A strategy for linking QTL maps has been developed. This database-enabled approach employs standardized plant ontology terms to categorize phenotypes and

Table 1 Mapping history of a representative gene interval on chromosome 2: *liguleless1 (lg1) - glossy2 (gl2) - booster1 (b1)*

Year	Map positions (cM)			Interval				Loci in map		
	lg1	*gl2*	*b1*	*lg1-gl2*		*gl2-b1*				
				cM	Named loci	cM	Named loci	Named	Total	Prepared by (in)
1935	0	19	38	19	0	19	0	62	62	M. Rhoades (Emerson et al., 1935)
1942	11	30	49	19	0	19	0	68	68	M. Rhoades (Rhoades, 1942)
1942	11	30	49	19	0	19	1	86	86	C. Burnham (Hayes and Immer, 1942)
1950	11	30	49	19	0	19	0	89	89	M. Rhoades (Rhoades, 1950)
1955	11	30	49	19	0	19	0	94	94	M. Rhoades (Rhoades, 1955)
1960	11	30	49	19	1	19	1	155	155	M.G. Neuffer (Neuffer, 1960)
1968	11	30	49	19	1	19	1	166	166	M.G. Neuffer (Neuffer et al., 1968)
1975	11	30	49	19	1	19	2	187	187	M.G. Neuffer & E. Coe (Neuffer and Coe, 1975)
1978	11	30	49	19	1	19	1	196	196	E. Coe & M.G. Neuffer (Coe and Neuffer, 1977)
1988	11	30	49	19	0	19	1	393	393	E. Coe et al. (Coe et al., 1988)
1993	11	30	49	19	0	19	5	559	559	E. Coe (Coe, 1993)
1995	30	52	71	22	2	19	7	668	668	E. Coe (Coe et al., 1995)
1997	30	52	71	22	5	19	9	843	843	M.G. Neuffer et al. (Neuffer et al., 1997)
2005	13.7	38	58	24.3	3	20	8	744	2449	E. Coe & M. Schaeffer (Genetic 2005; Coe and Schaeffer, 2005)
2005	13.03	30.6	-	17.57	3	-	-	983	30,387	M. Schaeffer (IBM2 2005 Neighbors Frame; Schaeffer et al., 2006)
2005	13.03	30.6	-	17.57	3	-	-	1,076	33,958	M. Schaeffer (IBM2 2005 Neighbors; Schaeffer et al., 2006)
2007	11.75	30.52	49.26	18.77	6	18.74	4	720	27,628	M. Schaeffer (IBM2 FPC0507; Schaeffer et al., 2006)
2007	11.75	30.52	49.3	18.77	*	18.78	*	1,278	3,210	E. Coe (Genetic 2007, in preparation)

* not yet compiled.

takes advantage of MaizeGDB as the central repository of genotypic and phenotypic data (Schaeffer, 2006). Although QTL results from any given study pertain only to a specific mapping population, compilation of results from multiple studies allows better understanding of the inheritance of that trait.

5.2 *Genetic to Cytological*

A number of strategies have been developed for linking the genetic and cytological maps. One of the earliest was the use of reciprocal A-A translocations. Translocations were essential in developing the 1935 maps for (a) associating chromosomes to linkage groups, (b) orienting linkage groups, and (c) providing supplemental information on the order of genes (Emerson et al., 1935; Rhoades and McClintock, 1935). Although data from at least 14 translocations were applied, only two were shown on the map (Fig. 1), inasmuch as recombination percentages around translocation breakpoints are reduced and cannot be related reliably to gene-to-gene distances. These 14 translocations were among 89 characterized by Anderson (1935) and used for chromosome placement and mapping in subsequent years. That set has since expanded to 1100 (data from MaizeGDB), a resource that provides up to 2200 breakpoint markers whose cytological coordinates are defined and whose genetic and physical map positions can continue to be useful aids to research.

Two other types of translocations can be used to place mutations or traits to chromosome arm. A set of translocations marked with recessive *waxy1* on chromosome 9 or *sugary1* on chromosome 4 allows association of recessives or dominants to chromosome with as few as 9 or 10 simple F2 progenies (Anderson, 1945). B-A translocations allow placement of recessive traits to narrower chromosomal regions (Roman, 1947; Beckett, 1991). The advantage of this method is that F1 progeny reveal the trait location due to the deficiencies for chromosome arm segments that are produced by these translocations. For both of these translocation methods, additional subsequent mapping with other markers is needed to refine map location.

Oat-maize addition lines offer another way to localize a trait or molecular polymorphism to chromosome arm or segment. Oat-maize addition lines have been made by crossing oat and maize and recovering oat lines that retain one maize chromosome. Using a PCR-based assay, any maize sequence can be localized to chromosome arm by screening for presence or absence of an amplified product in each of the oat-maize lines (Okagaki et al., 2001). Radiation hybrid derivatives of these lines enable more precise localization to specific chromosomal segment (Kynast et al., 2004).

Two other methods have been useful. Fluorescence in situ hybridization (FISH) has been used extensively to localize genes to chromosomes (Koumbaris and Bass, 2003; Kato et al., 2005; Lamb et al., 2007). Recombination nodule maps make it possible to predict the physical positions of genetic markers and to examine the distribution of markers across the maize chromosomes (Anderson et al., 2004; Anderson et al., 2006). A new tool, Morgan2McClintock, integrates recombination nodule and genetic maps to predict the chromosomal distance between genetically mapped markers (Lawrence et al., 2006).

5.3 *Genetic to Physical to Genome Sequence*

Anchoring of genetic and physical maps with common markers provides (a) association of physical map elements (BACs) with genetic points on the chromosomes, (b) orientation and ordering of physical-element assemblies (contigs), and (c) a framework skeleton for defining a minimum tiling path for sequencing.

Applying genetic map information to aid genome sequencing requires integrating genetic and physical maps. Moreover, the genetic map must be of high enough resolution such that recombination distances separating loci are on the scale of a few BAC lengths. The IBM population was created to meet the need for high resolution (Lee et al., 2002) and served as the foundation for the IBM map constructed by the Maize Mapping Project using data from public and private-sector collaborators.

Concomitant with development of the IBM genetic map, a physical map was constructed using fingerprint contig assembly of BAC clones from three libraries made from the B73 inbred line (Tomkins et al., 2002). Two methods were used to fingerprint the BAC clones. An agarose fingerprinting method resulted in 292,201 fingerprints that were automatically assembled into 4,518 contigs using FPC (Soderlund et al., 1997). High information content fingerprinting generated 350,253 fingerprints that were automatically assembled into 1,500 FPC contigs (Nelson et al., 2005).

A total of 25,908 markers were integrated into the FPC map (Wei et al., 2007). This included 1,902 genetically mapped markers (SSRs, RFLPs, SNPs and InDels) and 24,006 sequence-based markers (ESTs, BAC ends, and 40-bp overlapping oligonucleotide overgo probes). Associating markers to BACs involved three basic strategies. The first was hybridization of BAC libraries arrayed on filters with a suite of probes, including genetically mapped RFLPs (Yim et al., 2002), overgo probes derived from a maize EST unigene set (Gardiner et al., 2004), and overgo probes derived from sequences that had been genetically mapped in maize, sorghum and other grasses (e.g., Draye et al., 2001). The second strategy involved generation of BAC pools by six-dimensional pooling of a portion of one of the BAC libraries–representing six genome equivalents–and screening by PCR with primer pairs derived from single-copy genetically mapped sequences (Yim et al., 2007). The third strategy was sequencing of BAC ends (Messing et al., 2004). After manual editing, the final FPC map contained 721 contigs covering 2,150 Mb (93.5% of the total genome); 421 of the contigs (86.1% of the total genome) are anchored to the genetic map. The integrated map can be accessed at http://www.genome.arizona.edu/fpc/maize.

The FPC map provided the foundation for selecting approximately 19,000 BACs to make up a minimal tiling path for DNA sequencing. Details about the Maize Genome Sequencing Project can be viewed at http://maizesequence.org/overview.html.

6 The Future of Genetic Mapping

Emerging genomic sequence information is paving the way to an improved genetic map. The extraordinary potential of having a sequenced genome will only be realized when targeted traits defined by observation, measurement, or response can be

associated with the sequence. Accordingly, advancement of trait analysis requires that markers, annotated functional genes, and the sequence of the genome become tied to trait polymorphisms so that their genetic bases can be determined. These facts call for refined genetic maps, densely populated with markers that are usable in trait-mapping experiments and also placed physically on the genome sequence. Sequencing of the genome has reached the point that over 95% of genes for which there is a sequence can be placed on the genetic-map framework (Coe, personal observation). Applying such mapping *in silico* (sequenced gene to physical map to genetic map), a genetic map is in preparation and will be presented in MaizeGDB. This map uses IBM2 as the framework for genetic positions, has distances adjusted to standard centimorgans, applies the physical map for accretion of further genes, and places other genes on the basis of retrospective data from previous maps.

Finally, it should be noted that strong interest in trait mapping continues, with increasingly diverse materials, and can be expected to produce more mapping populations, genetic placement of a greater and greater range of traits, and higher resolution of trait variations. A deepening resource of information about maize as a research model and as a malleable crop plant will emerge from map-based analyses.

References

Anderson, E. (1935) Chromosomal interchanges in maize. *Genetics* **200**: 70–83.

Anderson, E. (1945) The following tables are compiled for the benefit of those using or wanting to use the sugary and waxy series of translocations for the study of economic or other characters in maize. *MNL* **19**: 5–8.

Anderson, L. K., A. Lai, S. M. Stack, C. Rizzon and B. S. Gaut (2006) Uneven distribution of expressed sequence tag loci on maize pachytene chromosomes. *Genome Res.* **16**: 115–122.

Anderson, L. K., N. Salameh, H. W. Bass, L. C. Harper, W. Z. Cande, G. Weber and S. M. Stack (2004) Integrating genetic linkage maps with pachytene chromosome structure in maize. *Genetics* **166**: 1923–1933.

Basten, C., B. S. Weir and Z.-B. Zeng (1997) QTL Cartographer: A reference manual and tutorial for QTL mapping. http://statgen.ncsu.edu/qtlcart/. Raleigh, N.C., Department of Statistics, North Carolina State University.

Beckett, J. B. (1991) Cytogenetic, genetic and plant breeding applications of B-A translocations in maize. In *Chromosome Engineering in Plants: Genetics Breeding, Evolution*. (P. Gupta and T. Tsuchiya, ed.) Elsevier Science Publishers, New York, pp. 493–529.

Bennetzen, J. L. and M. Freeling (1997) The unified grass genome: Synergy in synteny. *Genome Res.* **7**: 301–306.

Bi, I. V., M. D. McMullen, H. Sanchez-Villeda, S. Schroeder, J. Gardiner, M. Polacco, C. Soderlund, R. Wing, Z. Fang and E. H. Coe (2006) Single nucleotide polymorphisms and insertion-deletions for genetic markers and anchoring the maize fingerprint contig physical map. *Crop Sci.* **46**: 12–21.

Bortiri, E., G. Chuck, E. Vollbrecht, T. Rocheford, R. Martienssen and S. Hake (2006a) *ramosa2* encodes a LATERAL ORGAN BOUNDARY domain protein that determines the fate of stem cells in branch meristems of maize. *Plant Cell* **18**: 574–585.

Bortiri, E., D. Jackson and S. Hake (2006b) Advances in maize genomics: the emergence of positional cloning. *Curr. Op. Plant Biology* **9**: 164–171.

Buckler, E. S., B. S. Gaut and M. D. McMullen (2006) Molecular and functional diversity of maize. *Curr. Op. Plant Biology* **9**: 172–176.

Burr, B. and F. A. Burr (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. *Trends Genet.* **7**: 55–60.

Burr, B., F. A. Burr, K. H. Thompson, M. C. Albertson and C. W. Stuber (1988) Gene mapping with recombinant inbreds in maize. *Genetics* **118**: 519–526.

Carson, C., J. Robertson and E. Coe (2004) High-volume mapping of maize mutants with simple sequence repeat markers. *Plant Mol. Biol. Rep.* **22**: 131–143.

Casa, A., C. Brouwer, A. Nagel, L. Wang, Q. Zhang, S. Kresovich and S. Wessler (2000) The MITE family heartbreaker (Hbr): molecular markers in maize. *Proc. Natl. Acad. Sci. USA* **97**: 10083–10089.

Casa, A., A. Nagel and S. Wessler (2004) MITE display. *Methods Mol. Biol.* **260**: 175–188.

Chuck, G., A. Cigan, K. Saeteurn and S. Hake (2007) The heterochronic maize mutant *Corngrass1* results from overexpression of a tandem microRNA. *Nat. Genet.* **39**: 544–549.

Coe, E. (1993) Gene list and working maps. *MNL* **67**.

Coe, E., K. Cone, M. McMullen, S. S. Chen, G. Davis, J. Gardiner, E. Liscum, M. Polacco, A. Paterson, H. Sanchez–Villeda, C. Soderlund and R. Wing (2002) Access to the maize genome: An integrated physical and genetic map. *Plant Physiol.* **128**: 9–12.

Coe, E., D. J. Hancock, S. Kowalewski and M. Schaeffer (1995) Gene list and working maps. *MNL* **69**: 191–256.

Coe, E. and M. Neuffer (1977) The genetics of corn. In: *Corn and Corn Improvement.* (G. Sprague, ed.) American Society of Agronomy, Madison, WI, pp. 111–223.

Coe, E., M. Neuffer and D. A. Hoisington (1988) The genetics of corn. In: *Corn and Corn Improvement.* (G. Sprague and J. Dudley, ed.) American Society of Agronomy, Madison, WI, pp. 81–258.

Coe, E. and M. Schaeffer (2005) Genetic, physical, maps, and database resources for maize. *Maydica* **50**: 285–303.

Cone, K. C., M. D. McMullen, I. V. Bi, G. L. Davis, Y. S. Yim, J. M. Gardiner, M. L. Polacco, H. Sanchez-Villeda, Z. W. Fang, S. G. Schroeder, S. A. Havermann, J. E. Bowers, A. H. Paterson, C. A. Soderlund, F. W. Engler, R. A. Wing and E. H. Coe (2002) Genetic, physical, and informatics resources for maize: on the road to an integrated map. *Plant Physiol.* **130**: 1598–1605.

Davis, G. L., M. D. McMullen, C. Baysdorfer, T. Musket, D. Grant, M. Staebell, G. Xu, M. Polacco, L. Koster, S. Melia-Hancock, K. Houchins, S. Chao and E. H. Coe (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. *Genetics* **152**: 1137–1172.

Devos, K. M. and M. D. Gale (1997) Comparative genetics in the grasses. *Plant Mol. Biol.* **35**: 3–15.

Draye, X., Y. Lin, X. Qian, J. E. Bowers, G. Burow, P. Morell, D. Peterson, G. Presting, S. Ren, R. Wing and A. Paterson (2001) Toward integration of comparative genetic, physical, diversity, and cytomolecular maps for grasses and grains, using the sorghum genome as a foundation. *Plant Physiol.* **125**: 1325–1341.

Emerson, R., G. Beadle and A. Fraser (1935) A summary of linkage studies in maize. *Cornell Univ. Agric. Exp. Stn. Memoir* **180**: 1–83.

Evola, S., F. A. Burr and B. Burr (1986) The suitability of restriction fragment length polymorphisms as genetic markers in maize. *Theor. Appl. Genet.* **71**: 765–771.

Feuillet, C. and B. Keller (2002) Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. *Ann. Bot.* **89**: 3–10.

Fu, Y., T. J. Wen, Y. I. Ronin, H. D. Chen, L. Guo, D. I. Mester, Y. J. Yang, M. Lee, A. B. Korol, D. A. Ashlock and P. S. Schnable (2006) Genetic dissection of intermated recombinant inbred lines using a new genetic map of maize. *Genetics* **174**: 1671–1683.

Gardiner, J., S. Schroeder, M. L. Polacco, H. Sanchez-Villeda, Z. W. Fang, M. Morgante, T. Landewe, K. Fengler, F. Useche, M. Hanafey, S. Tingey, H. Chou, R. Wing, C. Soderlund and E. H. Coe (2004) Anchoring 9,371 maize expressed sequence tagged unigenes to the bacterial artificial chromosome contig map by two-dimensional overgo hybridization. *Plant Physiol.* **134**: 1317–1326.

Gardiner, J. M., E. H. Coe, S. Melia-Hancock, D. A. Hoisington and S. Chao (1993) Development of a core RFLP map in maize using an immortalized-F2 population. *Genetics* **134**: 917–930.

Hayes, H. and F. Immer (1942). *Methods of Plant Breeding*, McGraw-Hill, New York.

Helentjaris, T., M. Slocum, S. Wright, A. Schaefer and J. Nienhuis (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Theor. Appl. Genet.* **72**: 761–769.

Helentjaris, T., D. Weber and S. Wright (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. *Genetics* **118**: 353–363.

Kato, A., J. Vega, F. Han, J. Lamb and J. Birchler (2005) Advances in plant chromosome identification and cytogenetic techniques. *Curr. Op. Plant Biology* **8**: 148–154.

Kim, S. and A. Misra (2007) SNP genotyping: technologies and biomedical applications. *Annu. Rev. Biomed. Eng.* **9**: 289–320.

Koumbaris, G. and H. W. Bass (2003) A new single-locus cytogenetic mapping system for maize (*Zea mays* L.): overcoming FISH detection limits with marker-selected sorghum (*S. propinquum* L.) BAC clones. *Plant J.* **35**: 647–659.

Kynast, R. G., R. J. Okagaki, M. W. Galatowitsch, S. R. Granath, M. S. Jacobs, A. O. Stec, H. W. Rines and R. L. Phillips (2004) Dissecting the maize genome by using chromosome addition and radiation hybrid lines. *Proc. Natl. Acad. Sci. USA* **101**: 9921–9926.

Lai, J. S., Y. B. Li, J. Messing and H. K. Dooner (2005) Gene movement by Helitron transposons contributes to the haplotype variability of maize. *Proc. Natl. Acad. Sci. USA* **102**: 9068–9073.

Lamb, J., T. Danilova, M. Bauer, J. Meyer, J. Holland, M. Jensen and J. Birchler (2007) Single-gene detection and karyotyping using small-target fluorescence *in situ* hybridization on maize somatic chromosomes. *Genetics* **175**: 1047–1058.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. Daley, S. Lincoln and L. Newburg (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**: 174–181.

Lawrence, C., T. Seigfried, H. Bass and L. K. Anderson (2006) Predicting chromosomal locations of genetically mapped loci in maize using the Morgan2McClintock translator. *Genetics* **172**: 2007–2009.

Lee, M., N. Sharopova, W. D. Beavis, D. Grant, M. Katt, D. Blair and A. Hallauer (2002) Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Mol. Biol.* **48**(5): 453–461.

Lincoln, S., M. Daley and E. S. Lander (1992) Mapping genes controlling quantitative traits. http://www.broad.mit.edu/genome_software/other/qtl.html, Whitehead Institute Technical Report. **2007**.

Manly, K. F. and J. M. Olson (1999) Overview of QTL mapping software and introduction to map manager QT. *Mammal. Genome* **10**: 327–334.

McMullen, M. (2003) Quantitative trait locus analysis as a gene discovery tool. In: *Methods in Molecular Biology: Plant Functional Genomics Methods and Protocols*. (E. Grotewold, ed.) Humana Press, Inc., Totowa, NJ, **236**: pp. 141–154.

Messing, J., A. K. Bharti, W. M. Karlowski, H. Gundlach, H. R. Kim, Y. Yu, F. S. Wei, G. Fuks, C. A. Soderlund, K. F. X. Mayer and R. A. Wing (2004) Sequence composition and genome organization of maize. *Proc. Natl. Acad. Sci. USA* **101**: 14349–14354.

Messing, J. and H. K. Dooner (2006) Organization and variability of the maize genome. *Curr. Op. Plant Biology* **9**: 157–163.

Michelmore, R. W., I. Paran and R. V. Kessell (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segegrating populations. *Proc. Natl. Acad. Sci. USA* **88**: 9828–9832.

Moore, G., K. M. Devos, Z. Wang and M. D. Gale (1995) Cereal genome evolution - grasses, line up and form a circle. *Curr. Biol.* **5**: 737–739.

Morgante, M., S. Brunner, G. Pea, K. Fengler, A. Zuccolo and A. Rafalski (2005) Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize. *Nat. Genet.* **37**: 997–1002.

Morgante, M. and F. Salamini (2003) From plant genomics to breeding practice. *Curr. Op. Biotech.* **14**: 214–219.

Nelson, W. M., A. K. Bharti, E. Butler, F. S. Wei, G. Fuks, H. Kim, R. A. Wing, J. Messing and C. Soderlund (2005) Whole-genome validation of high-information-content fingerprinting. *Plant Physiol.* **139**: 27–38.

Neuffer, M. (1960) Linkage maps of maize chromosomes. *MNL* **40**: 167–172.

Neuffer, M. and E. Coe, Jr (1975) Corn (Maize). In: *Handbook of Genetics*. (R. King, ed.) Plenum Press, New York, **2**: pp. 3–30.

Neuffer, M., L. Jones and M. Zuber (1968). *The Mutants of Maize*. Crop Science Society of America, Madison, WI.

Neuffer, M., E. Coe and S. Wessler (1997). *Mutants of Maize*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Okagaki, R. J., R. G. Kynast, S. M. Livingston, C. D. Russell, H. W. Rines and R. L. Phillips (2001) Mapping maize sequences to chromosomes using oat-maize chromosome addition materials. *Plant Physiol.* **125**: 1228–1235.

Rhoades, M. (1942) Inasmuch as the writer was assigned chromosome 2 he has from time to time collected additional data on the location of certain genes placed in the map by two-point tests. *MNL* **16**: 4.

Rhoades, M. (1950) Meiosis in maize. *J. Heredity* **41**: 58–67.

Rhoades, M. (1955) The cytogenetics of maize. In: *Corn and Corn Improvement*. (G. Sprague, ed.) Academic Press, New York: pp. 123–220.

Rhoades, M. and B. McClintock (1935) The cytogenetics of maize. *Bot. Rev.* **10**: 292–325.

Roman, H. (1947) Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. *Genetics* **320**: 391–409.

Salvi, S., G. Sponza, M. Morgante, D. Tomes, X. Niu, K. A. Fengler, R. Meeley, E. V. Ananiev, S. Svitashev, E. Bruggemann, B. Li, C. F. Hainey, S. Radovic, G. Zaina, J. A. Rafalski, S. V. Tingey, G. H. Miao, R. L. Phillips and R. Tuberosa (2007) Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus m maize. *Proc. Natl. Acad. Sci. USA* **104**: 11376–11381.

Sanchez-Villeda, H., S. Schroeder, M. Polacco, M. McMullen, S. Havermann, G. Davis, I. Vroh-Bi, K. Cone, N. Sharopova, Y. Yim, L. Schultz, N. Duru, T. Musket, K. Houchins, Z. Fang, J. Gardiner and E. Coe (2003) Development of an integrated laboratory information management system for the maize mapping project. *Bioinformatics* **19**: 2022–2030.

Schaeffer, M. (2006) Consensus quantitative trait maps in maize: a database strategy. *Maydica* **51**: 357–367.

Schaeffer, M., H. Sanchez-Villeda, M. McMullen and E. Coe (2006) IBM2 2005 Neighbors – 45,000 locus resource for maize. *Plant and Animal Genome Conference Abstracts* **XIV**: 200.

Sharopova, N., M. D. McMullen, L. Schultz, S. Schroeder, H. Sanchez-Villeda, J. Gardiner, D. Bergstrom, K. Houchins, S. Melia-Hancock, T. Musket, N. Duru, M. Polacco, K. Edwards, T. Ruff, J. C. Register, C. Brouwer, R. Thompson, R. Velasco, E. Chin, M. Lee, W. Woodman-Clikeman, M. J. Long, E. Liscum, K. Cone, G. Davis and E. H. Coe (2002) Development and mapping of SSR markers for maize. *Plant Mol. Biol.* **48**: 463–481.

Snape, J. (1988) The detection and estimation of linkage using doubled haploid or single seed descent populations. *Theor. Appl. Genet.* **76**: 125–128.

Soderlund, C., I. Longden and R. Mott (1997) FPC: a system for building contigs from restriction fingerprinted clones. *Comput. Appl. Biosci.* **13**: 523–535.

Stam, P. (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. *Plant J.* **3**: 739–744.

Taramino, G. and S. Tingey (1996) Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* **39**: 277–287.

Tomkins, J. P., G. Davis, D. Main, Y. Yim, N. Duru, T. Musket, J. L. Goicoechea, D. A. Frisch, E. H. Coe and R. A. Wing (2002) Construction and characterization of a deep-coverage bacterial artificial chromosome library for maize. *Crop Sci.* **42**: 928–933.

Tuberosa, R. and S. Salvi (2006) Genomics-based approaches to improve drought tolerance of crops. *Trends Plant Sci.* **11**: 405–412.

Vuylsteke, M., M. R, R. Antonise, E. Bastiaans, L. Senior, C. Stuber, A. Melchinger, T. Luebberstedt, X. Xia, P. Stam, M. Zabeau and M. Kuiper (1999) Two high-density AFLP linkage maps of *Zea mays* L. : analysis of distribution of AFLP markers. *Theor. Appl. Genet.* **99**: 921–935.

Wei, F., E. Coe, W. Nelson, A. K. Bharti, F. Engler, E. Butler, H. Kim, J. L. Goicoechea, M. Chen, S. Lee, G. Fuks, H. Sanchez-Villeda, S. Schroeder, Z. Fang, M. McMullen, G. Davis, J. E. Bowers, A. H. Paterson, M. Schaeffer, J. Gardiner, K. Cone, J. Messing, C. Soderlund and R. A. Wing (2007) Physical and genetic structure of the maize genome reflects its complex evolutionary history. *PLOS Genetics* **3**: 1254–1263.

Wendel, J. F., M. M. Goodman, C. W. Stuber and J. B. Beckett (1988) New isozyme systems for maize (*Zea mays* L.): aconitate dehydratase, adenylate kinase, NADH dehydrogenase, and shikimate dehydrogenase. *Biochem Genet* **26**: 421–445.

Winkler, C., N. Jensen, M. Cooper, D. Podlich and O. Smith (2003) On the determination of recombination rates in intermated recombinant inbred populations. *Genetics* **164**: 741–745.

Yim, Y., G. Davis, N. Duru, T. Musket, E. Linton, J. Messing, M. McMullen, C. Soderlund, M. Polacco, J. Gardiner and E. Coe (2002) Characterization of three maize bacterial artificial chromosome libraries toward anchoring of the physical map to the genetic map using high-density bacterial artificial chromosome filter hybridization. *Plant Physiol.* **130**: 1686–1696.

Yim, Y. S., P. Moak, H. Sanchez-Villeda, T. A. Musket, P. Close, P. E. Klein, J. E. Mullet, M. D. McMullen, Z. Fang, M. L. Schaeffer, J. M. Gardiner, E. H. Coe and G. L. Davis (2007) A BAC pooling strategy combined with PCR-based screenings in a large, highly repetitive genome enables integration of the maize genetic and physical maps. *BMC Genomics* **8**:47.